Kidney International, Vol. 29 (1986), pp. 10–20

Structure, function and regulation of Na,K-ATPase in the kidney

PETER L. JØRGENSEN

Institute for Physiology, Aarhus University, Aarhus, Denmark

All zones of mammalian kidney, except the papilla, are abundant sources of Na,K-ATPase. The inner stripe of the red outer medulla of rabbit or pig kidney served as the starting material for the first successful purification of the Na,K-pump or Na,K-ATPase [1]. In this tissue the basolateral cell membranes of the predominant structure, the medullary thick ascending limb of Henle (MAL), are tightly packed with Na,K-pump sites in a concentration exceeding 40 million sites per cell as estimated by [^{3}H]-ouabain binding [2]. The pure preparation has been an ideal object for studying organization of the protein in the membrane [3–5] and for establishing structure-function relationships [6]. The pump protein retains its assymmetric orientation in the membrane. Formation of two-dimensional crystals suitable for image analysis can be induced by incubation in vanadate solution [6–8]. Immunologically the renal Na,K-ATPase is identical to the Na,K-pump in other mammalian tissues, including that in human red cells. Antibodies to the pure renal Na,K-ATPase are therefore available for immunoprecipitation studies of biosynthesis of the pump and for identification of the protein in other mammalian cell systems.

Figure 1 illustrates how the Na,K-pump provides the driving force for widely different active transport processes in discrete nephron segments (compare [9]). This diversity of the transport processes does not reside within the pump per se. Despite the apparent heterogeneity, the function of the Na,K-pump remains the same in all nephron segments, that is, to maintain electrochemical gradients for sodium ($\Delta\mu$Na). They form the driving force for secondary active transport of nutrients such as glucose and aminoacids, of metabolites such as citrate or succinate, of ions like protons, calcium, phosphate or chloride. Na^+-gradients that are maintained by the Na,K-pump also provide energy for secretion of organic acids like PAH and penicillin or of the diuretics furosemide and bumethanide and for the concentration and dilution of the urine. Only in a short segment of the nephron, the collecting ducts, is it the primary purpose of the transcellular transport process to control the excretion of the substrate of the pump, Na^+.

The concentration of Na,K-pump sites and provision of metabolic energy determines the capacity of the transcellular transport, but the nature and direction of solute transport in discrete segments depend on the Na^+-coupled carriers and the membrane conductances. Proper control of the integrated transport systems requires precise coordination of Na,K-pump function with that of a number of other structures. In contrast to the detailed information available about the structure and function of the pure renal Na,K-pump, little is known about the structure of the carriers that mediates $\Delta\ \mu Na^+$-driven secondary active transport of other solutes. An equally important gap is the lack of information about the mechanisms for cellular or hormonal control of the tubular transport processes.

The purpose of this article is to discuss recent information about the structure and function of the Na,K-pump proteins and the control of pump functions in kidney tubules. The nature of E_1-E_2 transitions in the α-subunit and their relation to cation binding, occlusion, and translocation in the reaction cycle will be examined to identify rate-limiting steps and points for regulatory control of the pump turnover rate. Cellular and humoral mechanisms for regulation of Na,K-pumping in kidney tubules will be discussed relative to the results of new methods for determining the concentration of [^{3}H]-ouabain binding sites in isolated tubules.

Structure of the pure renal Na,K-pump

The purification procedure. Membranes from the inner stripe of the bright red outer medulla form the starting material. The principle is to keep the Na,K-pump embedded in the membrane while extraneous proteins are extracted by incubation with sodium dodecyl sulphate in the presence of ATP [13]. The membrane-bound Na,K-ATPase can be recovered in a singie isopycnic centrifugation in a zonal rotor. The preparation is 90 to 100% pure with respect to the content of the specific proteins, the α-subunit with M_r 93,000–106,000 and the β-subunit with M_r 36,000 plus carbohydrate and with respect to phosphorylation and binding of the inhibitors ouabain and vanadate [4].

Soluble and fully active $\alpha\beta$-units are prepared by mixing pure membrane-bound Na,K-ATPase with $C_{12}E_8$ in proper conditions [14]. The soluble $\alpha\beta$-units each bind one molecule of ATP [15] and reconstitute directly into phospholipid vesicles to catalyze active Na,K-transport at high rates [14].

Ultrastructure of the Na,K-pump. To our knowledge, the Na,K-pump was first observed as distinct surface particles by electronmicroscopy after negative staining of pure membrane-bound Na,K-ATPase [16, 17]. The membranes appear as rounded discs studded with a uniform population of particles (12,500/μm^2) with diameters up to 5 nm. The protein can also be

Received for publication June 12, 1985

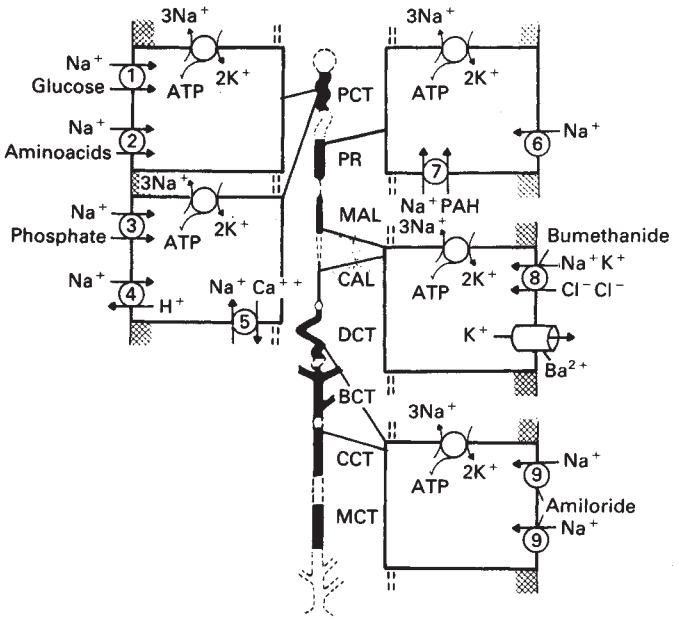

Fig. 1. *Model for primary active NA^+ transport coupled to ATP splitting by Na,K-ATPase and secondary active transport of solutes in coupling with Na^+ transport along its gradient ($\Delta\mu Na^+$).* Carriers mediating Na-coupled secondary active transport are placed in numerical order along the nephron. In PCT and PR carriers numbered 1-6 have been identified in isolated membrane vesicles [9]. In MAL and CAL transcellular NaCl transport consists of primary active Na-transport and secondary active Cl-transport coupled to Na-transport across lumen membrane by Na,K,Cl-cotransport system [10–12]. Recirculation of K^+ through the Ba-sensitive K-channel maintains the membrane potential and the driving force for flux of Cl^- from cytoplasm to blood.

visualized as intramembrane particles after freeze-fracture replication [16]. The orientation of the protein remains asymmetric in the pure membrane-bound Na,K-ATPase with particle-rich and -poor fracture faces. The distribution of particles is similar to that seen after fracture of basolateral cell membranes in the intact tubule cells of the MAL and in right-side-out vesicles of the plasma membrane [18]. In contrast, random orientation of Na,K-pump proteins is seen in reconstituted phospholipid vesicles prepared by mixing soluble Na,K-ATPase and excess phospholipid [19]. The intramembrane particles with a 9-nm diameter correspond to Na,K-pump molecules because the frequency of particles is proportional to the amount of Na,K-ATPase used in reconstitution and the density of particles is related linearly to the capacity for active Na-transport over a range of 0.2 to 16 particles per vesicle [19].

Molecular shape and subunit structure in membrane crystals. Na,K-pump molecules floating in the lipid bilayer are packed tightly in the disc-shaped membrane fragments of the pure preparation. The α-subunit in the lipid phase amounts to 6 to 8 mM corresponding to almost 1 g protein/ml of lipid phase. In these conditions of supersaturation, stabilization of the protein in the E_2-conformation in vanadate or phosphate medium favors formation of crystalline arrays. The protein particles associate in a two-dimensional lattice with bond energies that sufficiently compensate for the loss of translational and rotational entrophy of the freely moving particle. Within hours after the incubation begins, linear polymers (some of them dimeric)

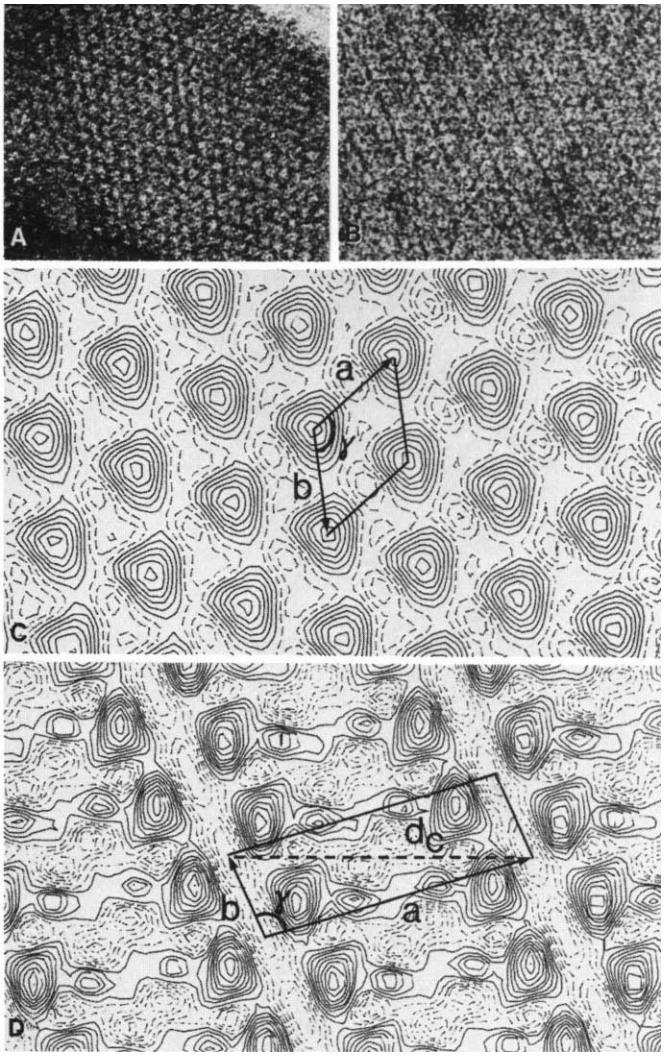

Fig. 2. *Two-dimensional crystals formed in vanadate* (**A**) *or in phosphate* (**B**)*, magnification (×410,000), and the corresponding computer-reconstructed images* (**C, D**). Selection of well-ordered crystalline arrays was made by optical diffraction. Images suitable for further analysis were densitometered at 20-μ intervals. Projection maps were calculated using the Fourier transform amplitudes and phases collected at the reciprocal lattice points [7]. The protein-rich regions (positive regions) are drawn with *unbroken contour lines* while negative stain regions have *dashed lines*. In the reconstructed images 1 mm corresponds to 2.8Å. The unit cell dimensions are in **C**: a = 53 Å, b = 51 Å, γ = 120°; and in d: a = 135 Å, b = 44 Å, γ = 101° [36].

are observed in the membranes. Later these polymers associate laterally to form extensive two-dimensional arrays [6, 8].

Computer-based image processing of electron micrographs of the negatively stained crystals shows that the symmetry in the vanadate crystal is p1[1] and that the unit cell contains one $\alpha\beta$-unit, Figure 2. The part of the particle protruding above the plane of the bilayer is a compact structure with a diameter close to 50 Å. The unit cell in the arrays grown in phosphate medium

[1] p1 and p21 indicate the lattice symmetry of the two-dimensional crystal using the nomenclature of the two-sided plane groups with one axis chosen to be perpendicular to the plane of the crystal [20].

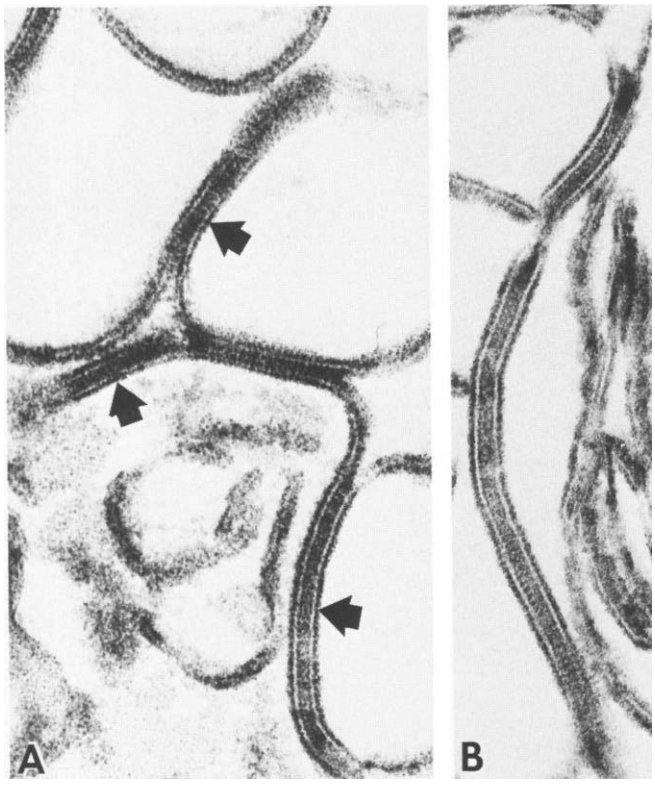

Fig. 3. *Thin sections of paired membranes produced by bathing the enzyme in crystallisation medium and then fixing the pellet with tannic-acid glutaraldehyde.* In **A** it is seen that a given membrane fragment can establish associations with several other fragments. In **B** is shown a transverse view with small particles at each side. The paired structures are formed through interaction of cytoplasmic protein protrusions of the Na,K-ATPase. (Reproduced with permission from [23]).

has two strong positive regions and contains two $\alpha\beta$-units. The symmetry is $p2_1^1$ and two $\alpha\beta$-units occupy one unit cell. These regions are subdivided into one large and one smaller peak possibly corresponding to α- and β-subunits, respectively [7]. Supporting this interpretation, tryptic cleavage of the α-subunit alters the shape of the body in the crystal while the smaller peak or hook is unaffected [21]. The hook representing the β-subunit can also be observed in monomer crystals when the resolution is sufficiently high [21]. The $\alpha\beta$-unit is therefore the minimum asymmetric unit of Na,K-ATPase in the membrane, but the protein crystallizes in two principally different forms, a monomer p1 and a dimer p21 form. It is probable that the dimer represents a transitory stage in the formation of the monomeric crystal since linear polymers consisting of paired protein units are seen during the formation of vandate-induced crystals.

Using the same techniques, the Ca-ATPase from sarcoplasmic reticulum also forms two-dimensional crystals [22]. In parallel to the larger molecular weight due to the presence of the β-subunit, the α-lattice dimensions of Na,K-ATPase is 40% larger than that of Ca-ATPase as it accommodates a hook-like structure which the Ca-ATPase lacks [1, 21].

The length of the Na,K-pump molecule. Formation of paired membranes in thin sections of the crystalline membrane-bound Na,K-ATPase, Figure 3, allows estimation of two important parameters of the Na,K-pump molecule, the total length of the molecule, 10.5 to 12.5 nm, and the length of extensions of the protein into the cytoplasmic and extracellular phases [23]. These structures are formed by protein-protein associations between cytoplasmic aspects of two membranes each composed of crystalline arrays of Na,K-ATPase. Assuming that the thickness of the bilayer between the phosophorous atoms is 4.8 nm, it can be calculated that the protein extends 5 nm beyond the cytoplasmic surface, but only 1 to 3 nm beyond the extracytoplasmic surface. These estimates agree with studies using labeling of the protein surface showing that the mass of the protein is asymmetrically distributed across the bilayer with three to four times more surface area on the cytoplasmic side of the membrane than on the extracellular side [24]. Fluorescence data also gave distances of 7 to 8 nm between binding sites for ATP at the cytoplasmic surface and the site for ouabain at the extracellular surface [3, 25].

Analysis of crystals thus provided the first direct observations of molecular shape, diameter, and length of the Na,K-pump. It should be remembered that negative staining reveals only hydrophilic features at the surface, while structural detail of lipid embedded portions or the path of polypeptide chains remain obscure. Further information requires elimination of the stain and the use of low-dose electronmicroscopy of more extensive and regular two-dimensional crystals. Another perspective is crystallization in three dimensions. Successful techniques are developed for small membrane proteins [26], but so far not for the large ion pump molecules.

Subunit structure. The $\alpha\beta$-unit is the minimum functional unit of pure renal Na,K-ATPase both in the membrane-bound form and in the soluble, fully active state in $C_{12}E_8$; but the possibility that formation of $(\alpha\beta)_2$-complexes is required for active Na,K-transport is not excluded. Each soluble $\alpha\beta$-unit binds one ATP molecule and performs the Na,K-ATPase reaction, but the stoichiometry of ATP binding in the membrane-bound state is more uncertain. Recent data show that soluble $\alpha\beta$-units undergo E_1-E_2 transitions and that α-α-subunit interaction need not be involved. This is consistent with the notion that a single $\alpha\beta$-unit can catalyze the whole series of intermediary reactions and cation transport reactions that normally occur in the membrane [27].

Disposition of the α-subunit and β-subunit in the membrane

Both the α- and the β-subunit are lipid-embedded and possess transmembrane segments [28]. The mass of α-subunit exposed on the cytoplasmic surface is three- to fourfold larger than that at the extracellular membrane surfaces [24]. In contrast most of the mass of the β-subunit including the carbohydrate is located at the extracellular surface. The path of the α-subunit in the membrane has been traced by determining the sidedness of cytoplasmic proteolytic splits and the localization of membrane-embedded segments relative to residues involved in formation of sites for ouabain and ATP at the two-membrane surfaces [4, 28]. In the Figure 4 model it is illustrated how the catalytic functions are executed in cytoplasmic domains that are separated by intramembrane segments.

Several attempts have been made to determine the amino acid sequence of α-subunit and β-subunit. Progress has been surprisingly slow, and only short sequences around the phosphorylation site and ATP binding area and the NH_2-terminus have been determined as yet [4]. The problem is that hydro-

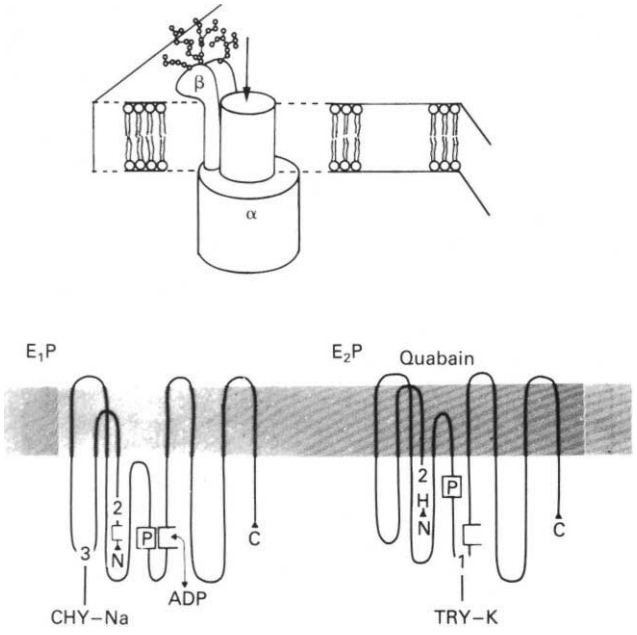

Fig. 4. *Model for the organization of α-subunit and β-subunit in the membrane* (above) *and for the path of the α-subunit protein chain in the membrane and the motion of the chain in E_1-E_2 transitions* (below). The *encircled numbers* mark the sites of primary tryptic cleavage in KCl (1) and (2) or NaCl (3) and chymotryptic cleavage in NaCl (3). In the E_1P conformation, bonds 2 and 3 are exposed to cleavage while bond 1 is protected. Transition to E_2P is accompanied by protonation of an ionizable group close to the NH-terminus. In the E_2P conformation, bond 3 is protected while bond 1 is exposed to trypsin and the position of bond 2 is such that it is cleaved secondary to cleavage of bond 1 within the same α-subunit. It is proposed that transition from E_1P to E_2P is accompanied by movement of a part of the segment containing the aspartyl phosphate from a relatively hydrophilic to a more hydrophobic environment. The segment between bonds 2 and 3 is proposed to engage in cation binding and formation of the pathway across the membrane.

phobic peptide chains precipitate when the protein is split for sequence analysis. It is likely that the aminoacid sequences will be determined on the basis of mRNA and DNA structures, but selection of the correct cDNA clone from a library demands prior knowledge of unique parts of the sequence.

Biosynthesis and assembly of the Na,K-pump protein

After a period of uncertainty several laboratories now find that the α-subunit is synthesized in membrane-bound polysomes and cotranslationally inserted into the membrane without a signal sequence [29, 30]. After labeling with [^{35}S]-methionine the α- and β-subunits are isolated from cultured cells and cell-free systems by immunoprecipitation with affinity purified antibodies. The unglycosylated β-subunit is detected as a chain with M_r 38,000 in cells treated with tunicamycin which blocks glycosylation in endoplasmic reticulum. After glycosylation in the endoplasmic reticulum, the molecular mass of the β-subunit reaches 45,000 daltons. Secondary stage glycosylation in the Golgi increases the molecular mass to about 60,000 daltons in a monensin-sensitive step [31]. The β-subunit is thought to be involved in integration and proper orientation of the α-subunit in the membrane, but the mechanism and site of assembly of the two proteins is not known.

Earlier studies suggested that the α-subunit was synthesized in free polysomes and discharged to the cytoplasm before insertion into the surface membrane [31, 32]. This would be the first case of an eukaryotic transmembrane polypeptide reaching the plasma membrane without prior cotranslational insertion in the endoplasmic reticulum. Post-translational insertion of the α-subunit would also differ from synthesis of sarcoplasmic reticulum Ca-ATPase in membrane-bound polysomes and cotranslational insertion without a signal sequence [33]. The Ca-pump protein shares numerous characteristics with the α-subunit of Na,K-ATPase, including molecular weight and sequence homologies around the sites for binding of ATP and phosphate.

Recent data suggest that the α-subunit undergoes transformation from an inactive to an active form while en route from the site of synthesis to the surface membrane. Pulse labeling with [^{35}S]-methionine in combination with photolabeling with NAB-ouabain [29] shows that newly synthesized Na,K-pump molecules are delivered to the cell surface with a $T_{1/2}$ of 50 min. If the cell membranes are disrupted before reaction with NAB-ouabain, it appears that newly synthesized Na,K-ATPase, before it reaches the cell surface, can bind ouabain supported by Mg^{2+} plus P_i, but not by Mg^{2+} plus ATP. The newly synthesized Na,K-pump thus seems to be blocked in an E_2-conformation that binds P_i and ouabain, but conveniently enough, it may be unable to assume the E_1-form and hydrolyze ATP before it reaches the cell surface.

Reaction mechanism of Na,K-pump

The E_1-E_2 conformational change is a large structural transition

The conformational change in the α-subunit was detected as a difference in exposure of bonds to tryptic cleavage in NaCl or KCl medium [34]. In addition to the bonds exposed to proteolysis, there is evidence that the structural change involves tryptophan residues [35], sulfhydryl groups, ionizable groups, and intramembrane segments [4]. Circular dichroism spectroscopy shows that Na,K-ATPase contains a roughly equal mixture of α-helical, β-sheet, and random coil structures [36]. The major structural transition between E_1 and E_2 involves an α-helix to β-sheet transition of about 7%. This is a relatively extensive change in secondary structure involving at least 80 aminoacid residues.

Relation of E_1-E_2 transition to cation exchange

The main question is whether this rather large motion in the protein is coupled to cation translocation. From 1975 to 1978, when these changes in protein structure were first described, the E_1-E_2 transitions detected by tryptic digestion [34] or fluorescence changes [35] were considered to be coupled to the actual movement of cation across the membrane, Na^+ extrusion to E_1P-E_2P and K^+ uptake to E_2-E_1 transition, as illustrated in the scheme in Figure 5. The dephosphoenzyme exchanges K^+ for Na^+ at the cytoplasmic surface and the phosphoenzyme exchanges Na^+ for K^+ at the extracellular surface, but these exchange reactions involve only minor perturbations of protein structure. These views gain strong support in a series of experiments on partial cation exchange reactions,

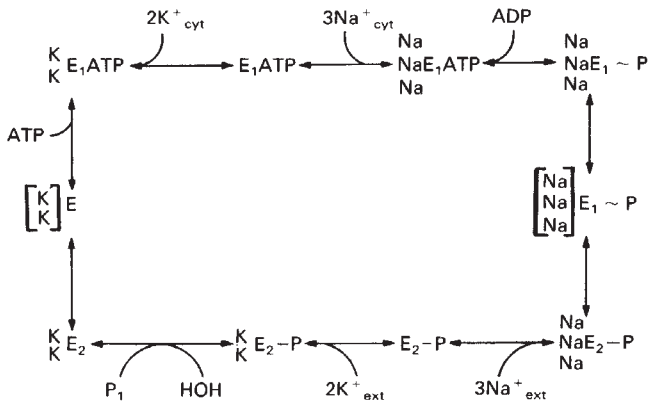

Fig. 5. *E_1-E_2 reaction cycle of the Na,K-pump with ping-pong sequential cation translocation.* Combination with ATP and release of ADP and P_i occur on the cytoplasmic surface. Cation sites are exposed to the cytoplasmic surface in E_1-forms. Binding of Na^+ is a condition for transfer of γ-phosphate from ATP to the aspartyl group in α-subunit. Phosphorylation is followed by occlusion of 3 Na^+ per phosphate group. In the E_2-form Na^+ cation sites are exposed to the extracellular medium where Na^+ is released and binding of K^+_{ext} is coupled to hydrolysis of the phosphoenzyme. Dephosphorylation is followed by occlusion of 2 K^+. Uptake of K^+ is associated with E_2-E_1 transition and accelerated by ATP. Na^+ and K^+ within *brackets* denote occluded ions.

selective proteolytic cleavage, cation binding and occlusion, and presteady-state ion fluxes.

Cation fluxes in absence of other ligands. In absence of ligands other than the monovalent cations, the Na,K-pump can be operated as a carrier mediating passive ouabain-sensitive fluxes in a direction opposite to that of the ATP-dependent active Na,K-transport [37]. As compared to the rate of the active Na,K-transport at 24°C, 30 to 40 Na/sec/α-subunit, the passive fluxes are slow, 0.1 Na/sec, but also in this case the conformational change in the protein is the rate-limiting step. The relative flux rates for a given cation of the series Rb^+, K^+, Cs^+, Na^+, or Li^+ correlate with the relative rates of the conformational transition E_2—E_1 in presence of that cation. The experiment therefore demonstrates that the cation induced transitions in absence of other ligands also are accompanied by translocation.

Cation binding and occlusion

In absence of other ligands Rb^+ or K^+ binding to cytoplasmic sites of Na,K-ATPase enter an occluded state within the molecule where they are unable to exchange with ions in the medium [11]. Also, in the course of the working cycle the Na,K-pump passes states in which Na^+ or K^+ are occluded. Rb^+ catalyzing hydrolysis of the phosphoenzyme enter the occluded state from the extracellular surface. Release of the occluded Rb^+ is regulated by ATP and P_i in the sense that they control the rate and the route by which Rb^+ leaves [38]. The rate in the forward direction of the cycle is increased by ATP and reversal is increased by P_i. This mechanism ensures tight coupling of cation flux to ATP hydrolysis and the rate and direction of pumping is determined by the relative concentrations of the ligands ATP and P_i.

The E_1P-form of the protein can occlude three Na ions per phosphorylation site [39]. Demonstration of the occluded form requires that the α-subunit is stabilized in the E_1P form by cleavage of bond 3 with chymotrypsin in NaCl medium [6]. The rate constants involved in occlusion and release of Na^+ from the occluded state are consistent with the view that occlusion of intracellular Na^+ in an (3Na)E_1P-form of phosphoenzyme and its release to the exterior after transition to the 3NaE_2P-form are responsible for translocation of Na^+ through the pump, as shown in Figure 5.

Na-Na and K-K-exchange and selective proteolytic cleavage. Selective proteolytic cleavage of bond 3 in NaCl or bond 1 in KCl (Fig. 4) has been a useful tool for examining the relationship between conformational change and cation translocation. The transitions between E_1P and E_2P are possible after cleavage of bond 1 with trypsin in KCl. This split does not interfere with either exchange reactions. In contrast cleavage of bond 3 with chymotrypsin in NaCl blocks the conformational transition from E_1P to E_2P and uncouples phosphoryl transfer from cation exchange [6]. The 1:1 isotope exchange of Na^+_{cyt} for Na^+_{ext} requires the presence of both ADP and ATP, and it is coupled to transition between E_1P and E_2P involving only the right-hand part of the scheme in Figure 5. The exchange of Rb^+_{cyt} for Rb^+_{ext} requires both ATP and P_i and the exchange is coupled to transitions between RbE_1ATP and RbE_2P [40] through the left-hand part of the reaction scheme in Figure 5. The data illustrate the tight coupling of cation exchange reactions to transitions between E_1P and E_2P.

The reaction cycle. The relationships between ion movements, phosphoryl transfer, and E_1-E_2 transition are shown in the E_1-E_2 scheme in Figure 5. In the normal clockwise operation, the major structural transitions between E_1 and E_2 forms of the α-subunit constitute the cation translocation steps, Na-extrusion involving the E_1P-E_2P transitions and K-uptake the E_2K-E_1ATP transition. Na^+ and K^+ are transported in sequence, Na^+ first and then K^+ in a ping-pong, sequential reaction.

As alternatives to the E_1-E_2 scheme, Skou [41] and Plesner et al [42] have proposed reaction cycles where E_1P and E_2P are not intermediates in the Na,K-dependent reactions. They question whether transition rates of E_1P to E_2P are fast enough to account for release of P_i during turnover. The alternatives include models in which Na,K-exchange is simultaneous in contrast to the ping-pong, sequential reaction. From recent data it is concluded that phosphorylation of the α-subunit is a necessary condition for hydrolysis of ATP by Na,K-ATPase both in the presence of Na^+ alone and with Na^+ plus K^+ in the medium [43, 44] and that E_1P-E_2P transition rates at 500 s^{-1} are among the fastest reactions of the catalytic cycle [44].

Pre-steady state fluxes. Another important test of the E_1-E_2 scheme and its coupling to ping-pong ion translocation is the measurement of pre-steady-state fluxes of Na^+ and K^+ in single turnover cycles. Forbush [45] loaded right-side-out vesicles with caged ATP and initiated a single turnover of the pump with a flash of light. Karlish and Kaplan [45] measured ATP-dependent uptake of ^{22}Na in reconstituted vesicles at 0°. Both experiments show an initial burst of ^{22}Na uptake reflecting transfer in the first turnover of the Na,K-pump which is insensitive to K^+ at the extracellular surface. The time of appearance of the burst shows that Na-transport involves more steps than formation of E_1P and that Na^+ efflux is an early event in the pump cycle relative to K^+ influx. Thus, in the first

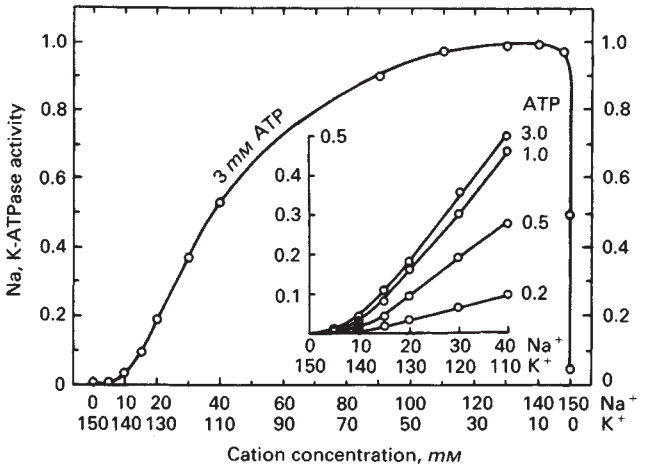

Fig. 6. *Activity of pure renal Na,K-ATPase as a function of Na^+, K^+, and ATP concentrations.* Initial rates of ATP hydrolysis were measured at the concentration of ATP and cations indicated in the graph in 3 mM $MgCl_2$, 30 mM histidine, pH 7.5, and 37°C. Isotonicity was maintained by exchanging NaCl and KCl.

turnover of the pump the ions are transported in sequence, Na^+ first and then K^+, consistent with the ping-pong, sequential mechanism of Na,K-transport.

Control of the rate of the reaction cycle

In the reaction cycle in Figure 5 the rate-limiting step is the conformational transition E_2-E_1, and this step seems also to be the control point of the reaction cycle both in vitro and in the tubule cells. At low concentrations of ATP, the rate-limiting steps are the transition from E_2K to E_1KATP and the release of K^+ from the occluded state. In conditions where cytoplasmic [K^+] increases or the substrate ATP is in short supply, this mechanism will slow the rate of ion movements. This is illustrated by the decrease in rate at a given Na/K ratio when ATP drops from 1 to 0.2 mM, Figure 6. A variation of ATP concentration in the range close to the cellular concentration of 1 to 3 mM has little influence on the rate of ATP splitting. In this range the rate is determined by the cytoplasmic Na/K ratio. At the relatively low ratios of 10Na/140K or 20Na/130K in the tubule cells [9], the data in Figure 6 predict that the molecular activity of the pump should be only 5 to 20% of the maximum, that is, 10 to 35 ATP/sec or 30 to 105 Na/sec. In this range of cation concentration the rate increases greatly with cytoplasmic [Na^+]. A doubling of the rate will be the consequence of even a small increase from 15 to 24 mM Na^+. In the next section these predictions from the behavior of isolated Na,K-ATPase will be compared with the actual molecular activities for Na^+ calculated from data in Table 1.

The concentration of Na,K-pumps in tubule cells

Turnover rates or molecular activities (sec^{-1}) for ATP and Na^+ can be calculated from Na,K-ATPase activities or Na-transport rates (pmoles/sec · cm) if the concentration of Na,K-pump sites (pmoles/cm) can be determined in microdissected segments. Micromethods that are sensitive enough to determine the number of [^{3}H]-ouabain binding sites in short microdissected nephron segments have been developed only recently [47]. The total [^{3}H]-ouabain binding was determined in medium containing vanadate and phosphate to stabilize the pump protein in the E_2P- or E_2V-form which traps ouabain with high affinity. Binding could be measured in the same number of discrete microdissected nephron segments, 4 to 7 pieces 0.2 to 1.2 mm long, as the Na,K-ATPase activity in direct assays using [32P]-ATP. [^{3}H]-ouabain binding was unaffected by premeabilization by osmotic and thermic shock while these maneuvers greatly increase Na,K-ATPase activity. This demonstrates that Na,K-ATPase is located in basal membranes with ouabain binding sites exposed at the membrane surface accessible from the tubule periphery or the blood side. This new technique for estimating the number of [^{3}H]-ouabain sites per tubule length in microdissected segments is particularly promising for assessing hormone effects on Na,K-ATPase. It will now be possible to determine if a change in the rate of Na-pumping is due to an effect of the hormone on the number of Na,K-pump sites or if it affects the molecular activity.

The data in Table 1 demonstrate that the concentration of pump sites show the same variation as previous estimates of Na,K-ATPase activities with very high values, 40 to 50 million sites/cell in distal convoluted tubule (DCT) and MAL. For comparison, there are about 500 Na,K-pump sites in a human red cell. In thin limbs of Henle, [^{3}H]-ouabain binding levels are very low and close to the sensitivity limit of the method. This confirms previous observations of very low Na,K-ATPase activities in the thin limbs, 0 in ATLH, and 0.3 to 0.7 pmoles/sec · cm in DTLH [48, 49].

Calculation of turnover for ATP or Na^+ shows that the molecular activity for ATP was variable, in a range between 30 and 161 sec^{-1} and generally higher in proximal than in distal tubular segments. The Na,K-ATPase activities are determined in media (Na, 50 mM; K, 5 mM; Mg, 10 mM; ATP, 10 mM) [50] or (Na, 100 mM; NH_4, 67 mM; Mg, 3.7 mM; ATP, 1.1 mM) [48] that are supposed to give maximum velocities of ATP hydrolysis. The large variation therefore suggests that demasking of latent activity may not be complete in all segments. Na transport rates are measured at the actual Na/K ratios and ATP concentrations prevailing in the cytoplasm of the isolated tubules. In Table 1 molecular activities for Na^+ in isolated tubules are shown to be within the range predicted from Figure 6 (30 to 105 Na/sec) for DCT and cortical collecting tubule (CCT), while they are two- to fourfold higher in per recta (PR) and cortical thick ascending limb of Henle (CAL). A likely explanation for this variation is that cytoplasmic Na/K ratios are higher in some trypsinized or microdissected tubules than in the native kidney cells.

Function and regulation of the Na,K-pump in proximal tubules

In PCT the active solute resorbtion is driven by the Na,K-pump, and it provides the osmotic gradient across the tubule that is the driving force for fluid transport [51]. There is a direct correlation between the rate of volume absorption and the difference in osmolality between luminal fluid and resorbate in proximal tubules. Data in Table 1 show that the activity of Na,K-ATPase and the concentration of [^{3}H]-ouabain binding sites per centimeter of proximal convoluted tubules (PCT) is 50 to 60% of those in MAL and DCT. Previous measurements on freeze-dried samples gave relatively low values for the activity

Table 1. Concentration of Na,K-pump sites and molecular activity of Na,K-ATPase in microdissected nephron segments from rabbit kidney[a]

Segment	[^{3}H]-ouabain binding[b]		Na,K-ATPase[c]	Na-transport[d]	Molecular activity	
	pmoles/cm	*sites/cell* × 10^{-6}	*pmoles/sec · cm*	*pmoles/sec · cm*	ATP/sec	Na/sec
PCT	0.109	25	7.6–17	19.5	70–161	179
PR	0.033	10	1.7–3.8	7.9	52–115	239
MAL	0.213	41	7.8–21	40	37–97	188
CAL	0.051	10	4.7–5.2	13.2	90–102	259
DCT	0.315	50	9.4–25	18.6	30–80	59
CCT	0.068	8	2.6–3.8	5.0	39–56	74
MCT	0.027	3	1.3–3.2		48–119	

Abbreviations: PCT, proximal convoluted tubule; PR, pars recta; MAL, medullary thick ascending limb of Henle; CAL, cortical collecting tubule; DCT, distal convoluted tubule; CCT, cortical collecting tubule; MCT, medullary collecting tubule.

[a] Molecular activity (sec^{-1}) was calculated by dividing Na,K-ATPase activities or Na-transport rates (pmoles/sec · cm) with capacities for [^{3}H]-ouabain binding (pmoles/cm). For calculation of the number of sites per cell, the binding capacity was divided by estimates of the number of cells per tubule length [12, 45].

[b] [47].

[c] [47, 48, 50].

[d] [48, 49].

per unit weight of PCT [52]. The recent data agree with estimates that the Na,K-pump is responsible for 33% of solute reabsorption in PCT [9]. In PCT Na^+ only enters the cytoplasm in coupling with other solutes, for example, glucose, aminoacids, and phosphate. In the first part of the PCT the rate of net solute reabsorption therefore depends on the load of glucose, aminoacids, phosphate, or other substrates for the Na-gradient driven carriers.

The maximum capacities for glucose and aminoacid resorbtion in isolated proximal tubules are close to or lower than the Na-transport capacity estimated from the content of Na,K-ATPase in PCT [9]. It is a common observation that the maximum rate of transport of phosphate decreases if the supply of glucose or aminoacids is increased. Pitts [53] suggested that these transport processes share a common step. Assuming that carriers for glucose, aminoacids, and phosphate are located in the same cells as illustrated in Figure 1, it is conceivable that the three substrates may compete for the transport energy and that the common step in the reabsorbtion process is the maintenance of $\Delta\mu Na^+$ by the Na,K-pump.

Regulation of NaCl transport in MAL

In segments with large capacities for Na-transport, precise cellular control of cation concentration and cell volume is required to protect the cell from extinction by flush through [54]. Relative to cell volume the MAL has a particularly high concentration of Na,K-pump sites and a high rate of active Na-transport, 40 pmoles/sec · cm tubule. In 1 cm of MAL the content of Na can be estimated to be about 177 pmoles Na^+/cm assuming that the cytoplasmic activity is 15 mM or 15 μmoles/cm^3. If in 1 sec 40 pmoles passes 1 cm MAL, it means that an amount equal to the total amount of cytoplasmic Na^+ will enter and leave the cell cytoplasm every 4 to 5 sec. The rate of Na-transport, 40 pmoles/cm · sec with 0.21 pmoles Na,K-pump sites/cm (Table 1) corresponds to 188 Na/sec per pump site. This is about one-third of the maximum molecular activity of the Na,K-pump. Considerable variations around this rate can be expected as a function of variations of the Na-load on the MAL. The extent to which this variation of pump rate involves changes in cytoplasmic ion activities is not known, but the cell possesses mechanisms for precise coordination of Na,K-pump rate with the Na^+ and K^+ conductances across lumen and basal membranes.

The important function of the thick ascending limb of Henle (TAL) in mammalian kidney is to dilute the tubule fluid by reabsorbing NaCl in excess of water and thus provide osmotic gradients for concentrating or diluting the urine. The epithelium has a remarkably low transepithelial osmotic water permeability and the reabsorption reduces the NaCl concentration in tubule fluid to a limiting value in the range 30 to 60 mM. The nature of the active NaCl transport has been the object of considerable controversy, but many investigators now agree that transcellular NaCl transport consists of primary active transport of Na^+ driven by the Na,K-pump and secondary active transport of Cl^- in coupling with entry of Na^+ across the lumen membrane [9]. Secondary active transport of Cl^- is coupled to transport of Na^+ by the Na,K,Cl-cotransport system [10, 12, 54]. The cotransporter is inhibited by cytoplasmic Na suggesting a negative feed-back control mechanism. As shown in Figure 1 it is assumed that K^+ recycles via a K-channel through the lumen membrane. This regulation of K-conductance is important for maintaining the membrane potential close to the magnitude of the equilibrium potential for K^+, thus providing a driving force for conductive flux of Cl^- from cytoplasm to blood [56]. A barium sensitive K-channel is present both in lumen and basal membranes and in secretory epithelia K-channels are stimulated by Ca^{2+} in the range 10^{-6} to 10^{-8} M [57].

A protein component of the Na,K,Cl-cotransport system with M_r 34,000 has recently been identified in membranes from MAL by covalent labeling with [^{3}H]-bumethanide [12]. The [^{3}H]-bumethanide binding cotransport system appears to be associated with actin and myosin-like cytoskeleton components. This association may be important for regulation of the rate of entry of ions across the lumen membrane. Both the Na,K,Cl-cotransport system and the barium-sensitive K-channel from MAL have been reconstituted in phospholipid vesicles together with the Na,K,Cl-cotransport system [58]. The Na,K,Cl-cotransport protein in MAL may also mediate hormone effects because it is identical to the hormone and cyclic

AMP-sensitive Na,K,Cl-cotransport system in avian red cells [54].

These observations open new experimental possibilities for investigations of the control mechanism of transcellular NaCl transport in MAL and other epithelia. The association of the Na,K,Cl-cotransport protein with cytoskeleton, the negative feedback control by Na^+ of the cotransport process and Ca-control of K-channels are elements in the control system. The relationship of these elements in the Na,K-pump is unknown, but together they form an integrated system for regulation of rapid transcellular NaCl transport without perturbing cell volume and ion activities.

Regulation of the concentration of Na,K-ATPase

Role of Na load. A diffuse loss of Na,K-pump sites is seen after adrenalectomy. It is more pronounced in the outer medulla than in cortex [59, 60], and it comprises multiple nephron segments [2, 48, 61, 62]. The decrease in Na,K-ATPase activity is due to a loss of Na,K-pump sites and not to a change in molecular activity [60, 62]. The rate of decline and the level of Na,K-ATPase after adrenalectomy depends on salt intake. A high NaCl intake can delay and partly prevent the decrease in Na,K-ATPase activity after adrenalectomy [59]. To explain this, it has been proposed that the activity of Na^+ or the Na/K ratio in cytoplasm can control the rate of biosynthesis or degradation of the enzyme protein [60].

The response of Na,K-ATPase to aldosterone injection also contrasts the segment specific distribution of hormone receptors. Although aldosterone receptors are absent from MAL [63, 64] Na,K-ATPase activity in MAL in outer medulla increases slowly after aldosterone injection, during 24 hr in parallel to restoration of the extracellular pool of Na^+ [60]. This change in activity was thought to be secondary to an increased Na-load due to reabsorption of Na^+ by Na,K-pump sites in epithelia with specific hormone receptors. Even in epithelia with specific hormone receptors the evidence that ion activities in cytoplasm may control the rate of biosynthesis deserves attention. The observation that amiloride blocks the increase in Na,K-ATPase activity that is seen 3 hr after aldosterone injection to adrenalectomized rabbits [65] suggests that the change in Na,K-ATPase activity is secondary to a change in rate of entry of Na^+ through amiloride-sensitive Na-channels in the lumen membrane.

The role of mineralocorticoids in regulation of Na,K-ATPase in CCT (Table 2)

Aldosterone augments the rate of active ouabain sensitive Na-transport across a number of epithelia bordering the extracellular fluid volume from the surroundings. Experimental data on isolated kidney CCT segments clearly illustrate the unsolved questions related to mineralocorticoid effects on Na,K-transport. In CCT, there is a high concentration of mineralocorticoid receptor sites as estimated by [^{3}H]-aldosterone binding ($7.1 \cdot 10^7$ receptor sites/cm [63]) and both short- and long-term changes in mineralocorticoid concentration in plasma are known to induce large changes in Na,K-pump rates in CCT.

As seen from Figure 7, the Na,K-ATPase activity in the CCT varies over a wide range, more than tenfold, as a function of long-term variations of mineralocorticoid hormone concentration in plasma. The Na,K-ATPase activity falls to 0.6 pmoles/sec · cm after adrenalectomy and may rise to 8 pmoles/sec · cm after administration of pharmacological doses of deoxycorticosterone [50, 54] or chronic variation of plasma aldosterone [56, 68]. The molecular mechanism behind these changes is still not known, but the recent data shown in Figures 7 and 8 provide answers to some old questions. Ultrastructural analysis, transport assay, and titration of the concentration of [^{3}H]-ouabain

Table 2. Summary of mineralocorticoid effects on Na,K-ATPase activities, [^{3}H]-ouabain binding, Na-transport and basal membrane areas in CCT of New Zealand rabbit kidney

	Adrenalectomized				
	Untreated	Aldosterone	Control	DOCA	Ref.
Principal cells					
Lumen membrane area, $\mu m^2/cm \cdot 10^5$			0.77	0.93	65
Basal membrane area, $\mu m^2/cm \cdot 10^5$			64	156	65
Na,K-ATPase activity, *pmoles/sec · cm*	0.59	1.54	1.8–2.6	8.2	48, 50
Na-transport, *pmoles/sec · cm*			7.1	17.1	67
[^{3}H]-ouabain binding, *pmoles/cm*	0.017	0.048	0.068		62

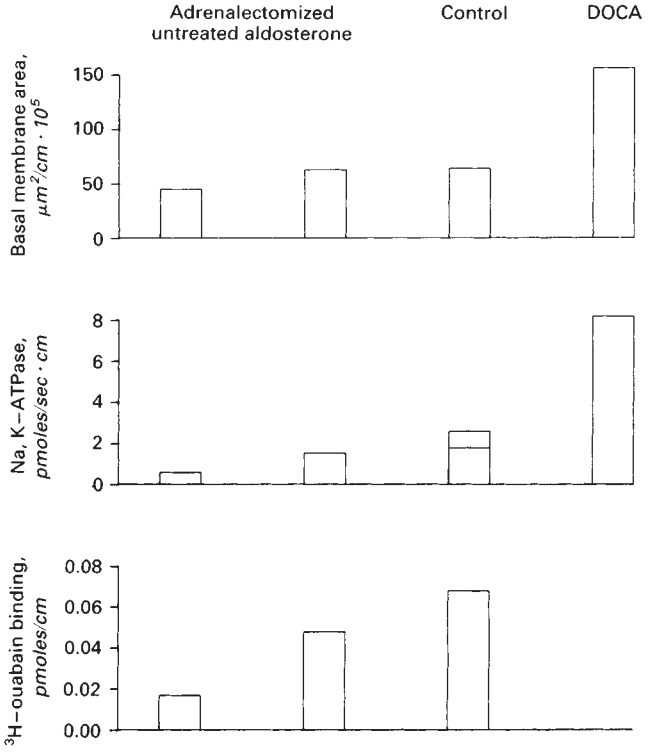

Fig. 7. *Summary of mineralocorticoid effects on Na,K-ATPase activities* [47, 49], *[3H]-ouabain binding* [46], *and basal membrane areas in principal cells of CCT* [65] *of New Zealand rabbit kidney.* Na-transport across the tubule varies in parallel with Na,K-ATPase activity from 7.1 pmoles/sec · cm in control rabbits to 17.1 pmoles/sec · cm in DOCA-treated rabbits [66], but measurements of Na-transport are not available for adrenalectomized rabbits.

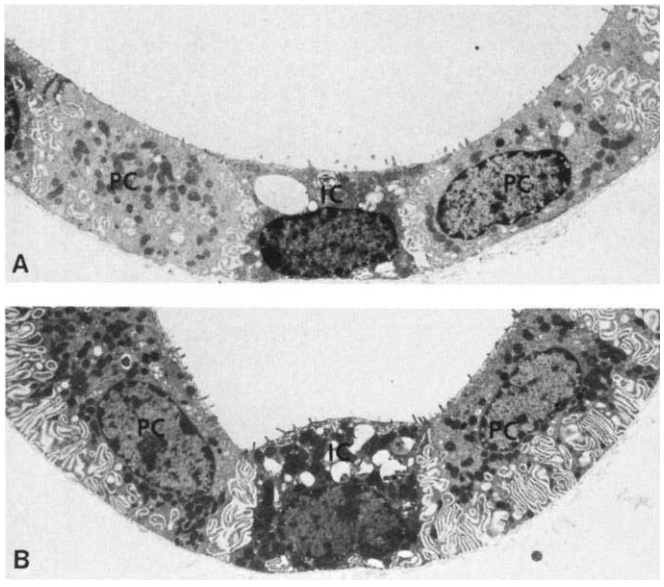

Fig. 8. *Effect of DOCA-treatment on the basal membrane area in principal cells in CCT from New Zealand rabbits;* **A)** *tubule from untreated control rabbit;* **B)** *tubule from rabbits treated daily with intramuscular injections of 5 mg DOCA for 11 to 18 days before sacrifice.* (Reproduced with permission from [65])

binding sites allow examination of the cellular localization of the changes and the variation in Na,K-pump concentration relative to basolateral membrane area.

Acute adaptation of Na,K-pump rates. In each of the regulatory conditions acute changes in Na-transport rate can be achieved by altering the molecular activity of existing Na,K-pumps without altering the concentration of pump sites in the tubule. In control conditions the Na-transport rate in CCT is 5 to 7 pmoles Na/cm · sec (Table 1 and Fig. 7). With 0.068 pmoles Na,K-pump sites/cm, this equals a molecular activity of 75 to 104 Na/sec, which corresponds to 14 to 20% of maximum molecular activity of the Na,K-pump. Theoretically, the Na-transport rate could be raised five- to sevenfold without altering the concentration of sites. This is a large reserve capacity for acute adaptation of the rate of existing pump sites, for example, in response to an increase in rate of entry of Na^+ across the lumen membrane in response to aldosterone as demonstrated in toad bladder [69].

Aldosterone effects after adrenalectomy. Short-term effects of aldosterone have only been demonstrated after adrenalectomy. Recent data show that there is a parallel change in Na,K-ATPase activity and [^{3}H]-ouabain binding capacity in CCT after adrenalectomy and after aldosterone injection [62]. This means that the molecular activity of the Na,K-pump is the same in each of the regulatory conditions. After adrenalectomy the concentration of Na,K-pump sites in CCT drops slowly to reach the new level after 1 week. In contrast to this, the response to aldosterone injections is relatively fast in some of the reports, but the rates differ considerably. There are several reports that aldosterone injection restores Na,K-ATPase activity in the CCT to the control level within 3 hr both in adrenalectomized rats [2, 56] and rabbits [65]. In rabbits both Na,K-ATPase activity and [^{3}H]-ouabain binding sites in CCT reached control levels 3 hr after aldosterone [62]. In recent experiments on adrenalectomized rats, aldosterone in same dose (1 μg/100 g day) increased the Na,K-ATPase activity in CCT more slowly from 48% to reach 73% of control activity after 24 hr, 106% after 3 days, and 170% after 7 days [70]. In adrenalectomized mice, the level of Na,K-ATPase activity is reduced to 40% of control after adrenalectomy, but it remained unaffected 1 to 3 hr after aldosterone injections [71].

The relatively rapid effect on the concentration of Na,K-pump sites in some experiments could suggest that aldosterone affects the rate of synthesis of Na,K-ATPase proteins directly to induce an acute increase in amount of enzyme. A direct effect of physiological concentrations of aldosterone at the transcriptional level to increase the amount of mRNA and the rate of synthesis of α-subunit of Na,K-ATPase has been demonstrated in toad bladder [72]. This effect is relatively slow, 20 hr, but it is insensitive to amiloride. At present the evidence is controversial, since a blocking effect of amiloride in mammalian kidney suggests that the effect of aldosterone on biosynthesis of Na,K-ATPase is secondary to an increase in rate of entry of Na^+ across the lumen membrane [65].

Mineralocorticoid effects on Na-transport, Na,K-ATPase and basolateral membrane area (Table 2)

Chronic changes in capacity for Na,K-pumping are elicited by physiological concentrations of aldosterone. As a function of variation in plasma aldosterone from 0 to 217 ng/100 ml the active Na-flux from lumen to bath in CCT shows a fourfold variation from 3.5 to 12 pmoles/sec · cm with a $K_{1/2}$ of 7 ng/100 ml aldosterone in plasma [67]. This increase in transport rate with increasing aldosterone concentration in plasma is accompanied by parallel amplification of basolateral membrane area of principal cells and Na,K-ATPase activity [68]. In this series enzyme activities are obtained in freeze-dried samples where activities generally are lower than those in Figure 7 and the data cannot be expressed in units per centimeter of tubule [68], but this effect of aldosterone is similar to that shown in Figure 7 for DOCA.

In rabbits, Na,K-ATPase activity drops slowly to reach levels about 20% of control after 1 week. It is seen from Figure 7 that the decrease in basal membrane area is more moderate suggesting a decrease in density of Na,K-pump sites per unit of membrane area after adrenalectomy. DOCA treatment induces a threefold increase in Na,K-ATPase activity. In parallel there is a 2.4-fold increase in Na-transport from lumen to bath in the isolated perfused CCT. The treatment greatly augments the area of basal membrane infoldings in the principal cells, but not in the intercalated cells of CCT [66] (Figs. 7 and 8). The morphometric measurements on sections shown in Figure 8 show that the basolateral membrane area in principal cells per centimeter of tubule is increased almost threefold, while the lumen membrane area remains constant. The density of Na,K-pump sites in basolateral membranes of the principal cells in CCT is close to a maximum value in the control situation (6,400 per μm^2) since the density is close to that in pure membrane-bound Na,K-ATPase (7 to 12.000 per μm^2). Measurements of [^{3}H]-ouabain binding sites are not available after DOCA treatment, but the high density of sites in the control situation suggests that a further increase in Na,K-ATPase activity must involve a parallel increase in number of Na,K-pump sites and membrane area with a constant density of pump sites in the newly synthesized membrane.

The threefold increase in Na,K-ATPase following DOCA treatment occurs gradually over about a week [50]. In control rabbits the levels of Na,K-ATPase and [^{3}H]-ouabain binding sites corresponds to about 0.12 ng pure Na,K-ATPase per centimeter of CCT or 8 million sites per cell [47]. The new level reached after 7 to 8 days of treatment with DOCA is 3.3-fold higher. The time course describing the approach to the new level of Na,K-ATPase activity after DOCA treatment allows calculation of the first order rate constant for degradation $k_d(time^{-1})$, and the rate of synthesis in the new steady-state, k_s(units/time · mass), where Na,K-ATPase activity, 8.2 pmoles/sec · cm will be equal to the ratio k_s/k_d [73]. The reported data give an average k_d of 0.19 + 0.04 day^{-1}. This value is close to previous estimates of the degradation rate constant [9].

Together the data provide convincing evidence for parallel changes in Na,K-ATPase activity, Na,K-transport, and basolateral membrane area in CCT as a function of plasma aldosterone concentration. The question now is if mineralocorticoids induce Na,K-ATPase and basolateral membrane selectively or if the hormone regulates the capacity of other systems in parallel to changes in capacity for Na,K-pumping. Parallel measurements of adenylate cyclase and Na,K-ATPase activities show that the variation in adenylate cyclase activity was moderate [50]. However, citrate synthetase activity seems to vary considerably [65], and it appears from the ultrastructural analysis of CCT in Figure 8 [66, 68] that the density of mitochondria in the principal cells is increased almost in parallel to the change in area of basolateral membrane infoldings after chronic increases of plasma aldosterone or DOCA treatment. Estimates of the number of amiloride-sensitive Na-channels of the lumen membrane of CCT are not available, but data from toad bladder suggest that changes in rate of entry of Na^+ can be due to altered density of Na-channels in lumen membrane. It is therefore probable that chronic adaptation of transcellular Na-transport capacity of CCT involves parallel changes in amount of Na,K-pumps, Na-channels and in capacity for ATP supply from mitochondria.

Acknowledgments

Laboratory investigations are supported by the Danish Medical Research Council and Novo's Foundation. I am grateful to Dr. A. Doucet, College de France, for making manuscripts with valuable information available prior to publication.

Reprint requests to Dr. P. L. Jørgensen, Institute of Physiology, Aarhus University, 8000 Aarhus C, Denmark

References

1. JØRGENSEN PL: Isolation and characterization of the components of the sodium pump. *Quart Rev Biophys* 7:239–274, 1975
2. MERNISSI GE, DOUCET A: Short-term effect of aldosterone on renal sodium transport and tubular Na-K-ATPase in the rat. *Pflügers Arch* 399:139–146, 1983
3. CANTLEY LC: Structure and mechanism of the (Na,K)-ATPase. *Curr Top Bioenergetics* 11:201–237, 1981
4. JØRGENSEN PL: Mechanism of the Na,K-ion pump. Protein structure and conformations of the pure Na,K-ATPase. BBA Reviews on Biomembranes. *Biochim Biophys Acta* 694:27–68, 1982
5. KYTE J: Molecular considerations relevant to the mechanism of active transport. *Nature* 292:201–204, 1981
6. JØRGENSEN PL, SKRIVER E, HEBERT H, MAUNSBACH AB: Structure of the Na,K-pump: Crystallization of pure membrane-bound Na,K-ATPase and identification of functional domains of the α-subunit. *Ann New York Acad Sci* 402:203–219, 1982
7. HEBERT H, JØRGENSEN PL, SKRIVER E, MAUNSBACH AB: Crystallization patterns of membrane-bound Na,K-ATPase. *Biochem Biophys Acta* 689:571–574, 1982
8. SKRIVER E, MAUNSBACH AB, JØRGENSEN PL: Formation of two-dimensional crystals in pure membrane-bound Na,K-ATPase. *FEBS Letters* 131:219–222, 1981
9. JØRGENSEN PL: Sodium and potassium ion pump in kidney tubules. *Physiol Rev* 60:864–917, 1980
10. FORBUSH B, PALFREY HC: [^{3}H]Bumetanide binding to membranes isolated from dog kidney outer medulla. *J Biol Chem* 258:11787–11792, 1983
11. GLYNN IM, RICHARDS DE: Occlusion of rubidium ions by the sodium-potassium pump: Its implication for the mechanism of potassium transport. *J Physiol* 330:17–43, 1982
12. JØRGENSEN PL, PETERSEN J, REES WD: Photolabeling with [^{3H}]-bumethanide of a NaCl-KCl-cotransport protein of M_r 34,000 from thick ascending limb of Henle's loop; the protein is associated with cytoskeleton components. *Biochim Biophys Acta* 775:105–110, 1984
13. JØRGENSEN PL: Isolation of (Na^+ + K^+)dependent ATPase. *Meth Enzymology* 32:277–290, 1974
14. BROTHERUS JR, JACOBSEN L, JØRGENSEN PL: Soluble and enzymatically stable Na,K-ATPase from mammalian kidney consisting predominantly of αβ-units. Preparation, assay and reconstitution of active transport. *Biochim Biophys Acta* 731:290–303, 1983
15. JENSEN J, OTTOLENGHI P: ATP binding to solubilized (Na^+ + K^+)-ATPase. The abolition of subunit-subunit interaction and the maximum weight of the nucleotide-binding unit. *Biochim Biophys Acta* 731:282–289, 1983
16. DEGUCHI N, JØRGENSEN PL, MAUNSBACH PL: Ultrastructure of the sodium pump: Comparison of thin sectioning, negative staining and freeze-fracture of purified membrane-bound (Na^+, K^+)ATPase. *J Cell Biol* 75:619–634, 1977
17. MAUNSBACH AB, JØRGENSEN PL: Ultrastructure of highly purified preparations of (Na^+ + K^+)ATPase from the outer medulla of rabbit kidney. *Proc Eight Int Congr Elect Microsc Australia* 2:214–215, 1974
18. SKRIVER E, MAUNSBACH AB, JØRGENSEN PL: Ultrastructure of Na,K-ATPase in plasma membranes and in reconstructed phospholipid vesicles. *Curr Top Membr Trans* 19:119–122, 1983
19. SKRIVER E, MAUNSBACH AB, JØRGENSEN PL: Ultrastructure of Na,K-transport vesicles reconstituted with purified renal Na,K-ATPase. *J Cell Biol* 86:746–754, 1980
20. FULLER SD: Three dimensional reconstruction of membrane protein crystals. *Meth Cell Biol* 22:251–296, 1981
21. MOHRAZ M, SMITH PR: Structure of (Na^+,K^+)-ATPase as revealed by electron microscopy and image processing. *J Cell Biol* 98:1836–1864, 1984
22. DUX L, MARTONOSI A: Two-dimensional arrays of proteins in sarcoplasmic reticulum and purified Ca^{2+}-ATPase vesicles treated with vanadate. *J Biol Chem* 258:2599–2603, 1983
23. ZAMPIGHI G, KYTE J, FREYTAG W: Structural organization of (Na^+ + K^+)-ATPase in purified membranes. *J Cell Biol* 98:1851–1864, 1984
24. SHARKEY RG: Lactoperoxidase catalyzed iodination of Na,K-ATPase in the MDCK epithelial cell line and canine renal membranes. *Biochim Biophys Acta* 730:327–341, 1983
25. MOCZYDLOWSKI EG, FORTES PAG: Characterization of 2′,3′-O-(2,4,6-trinitrocyclohexadienylidine)adenosine 5′-triphosphate as a fluorescent probe of the ATP site of sodium and potassium transport adenosine triphosphatase. Determination of nucleotide binding stoichiometry and ion-induced changes in affinity for ATP. *J Biol Chem* 256:2346–2356, 1981
26. MICHEL H, OSSTERHELT D, HENDERSON R: Orthothombic two-dimensional crystal form of purple membranes. *Proc Natl Acad Sci USA* 77:338–342, 1980
27. JØRGENSEN PL: Protein structure and conformational transitions in αβ-units by pure Na,K-ATPase, in "The sodium pump," *4th Int Conf on Na,K-ATPase*, edited by GLYNN IM, ELLORY JC, Cambridge, Company of Biologists, in press, 1984
28. JØRGENSEN PL, KARLISH SJD, GITLER C: Evidence for the organization of the transmembrane segments of Na,K-ATPase based on labelling lipid embedded and surface domains of the α-subunit. *J*

Biol Chem 257:7435–7442, 1982
29. CAPLAN MJ, PALADE GE, JAMIESON JD: Cell surface expression and activation of newly synthesized Na,K-ATPase in MDCK cells, in "The sodium pump," *4th Int Conf on Na,K-ATPase*, edited by GLYNN IM, ELLORY JC, Company of Biologists, Cambridge. In press, 1984
30. NABI N, SHERMAN J, SABATINI DD, MORIMOTO T: Biosynthesis of rat brain Na,K-ATPase. *J Cell Biol* 447, 1984
31. SHERMAN J, MORIMOTO T, SABATINI DD: Biosynthesis of Na,K-ATPase in MDCK cells. *Curr Top Membr Transp* 19:753–764, 1983
32. HIATT A, MCDONOUGH AA, EDELMAN IS: Assembly of Na,K-ATPase. Post-translational membrane integration of the α-subunit. *J Biol Chem* 259:2629–2635, 1984
33. MOSTOV KE, DEFOOR P, FLEISCHER S, BLOBEL G: Co-translational membrane integration of calcium pump protein without signal sequence cleavage. *Nature* 292:87–88, 1981
34. JØRGENSEN PL: Purification and characterization of Na^+,K^+-ATPase. V. Conformational changes in the enzyme. Transitions between the Na^+-form and the K^+-form studied with tryptic digestion as a tool. *Biochim Biophys Acta* 401:399–415, 1975
35. KARLISH SJD, YATES DW: Tryptophan fluorescence of Na,K-ATPase as a tool for study of the enzyme mechanism. *Biochim Biophys Acta* 527:115–130, 1978
36. GRESALFI TJ, WALLACE BA: Secondary structural composition of the Na,K-ATPase E_1 and E_2 conformers. *J Biol Chem* 259:2622–2628, 1984
37. KARLISH SJD, STEIN WD: Passive rubidium fluxes mediated by the Na,K-ATPase reconstituted into phospholipid vesicles. ATP and phosphate-free conditions. *J Physiol* 328:295–316, 1982
38. KARLISH SJD, STEIN WD: Effects of ATP or phosphate on passive rubidium fluxes mediated by Na-K-ATPase reconstituted into phospholipid vesicles. *J Physiol* 328:317–331, 1982
39. GLYNN IM, HARA Y, RICHARDS DE: The occlusion of sodium ions within the mammalian sodium-potassium pump: its role in sodium transport. *J Physiol* In press, 1984
40. GLYNN IM, KARLISH SJD: The sodium pump. *Ann Rev Physiol* 37:13–53, 1975
41. SKOU JC: The Na,K-activated enzyme system and its relationship to transport of Na and K. *Q Rev Biophys* 7:401–434, 1975
42. PLESNER IW, PLESNER L, NØRBY JG, KLODOS I: The steady-state kinetic mechanism of ATP hydrolysis catalyzed by membrane-bound Na,K-ATPase from ox brain. III. A minimal model. *Biochim Biophys Acta* 83:346–356, 1981
43. FORTES PAG, LEE JA: Phosphoenzyme forms sensitive to ADP and K are intermediates of active Na and K transport mediated by (Na,K)-ATPase. *J Biol Chem* 259:11176–11179, 1984
44. HOBBS AS, ALBERS RW, FROEHLICH JP: Effects of oligomycin on the partial reactions of the sodium plus potassium-stimulated adenosine triphosphatase. *J Biol Chem* 258:8163–8168, 1983
45. FORBUSH B: An apparatus for rapid kinetic analysis of isotopic efflux from membrane vesicles and of ligand dissociation from membrane proteins. *Anal Biochem* 140:495–505, 1984
46. KARLISH SJD, KAPLAN JH: Pre-steady state cation transport by the Na,K-pump, in "The sodium pump," *4th Int Conf Na,K-ATPase*, edited by GLYNN IM, ELLORY JC, Cambridge, Company of Biologists, in press, 1984
47. MERNISSI GE, DOUCET A: Quantitation of [^{3}H]-ouabain binding and turnover of Na-K-ATPase along the rabbit nephron. *Am J Physiol* 247:F158–F167, 1984
48. GARG L, KNEPPER M, BURG M: Mineralocorticoid stimulation of Na-K-ATPase in nephron segments. *Am J Physiol* 240:F536–F544, 1981
49. JACOBSON HR: Functional segmentation of the mammalian nephron. *Am J Physiol* 241:F203–F218, 1981
50. MERNISSI GE, CHABARDES D, DOUCET A, HUS-CITHAREL A, IMBERT-TEBOUL M, LE BOUFFANT F, MONTEGUT M, SIAUME S, MOREL F: Changes in tubular basolateral membrane markers after chronic DOCA treatment. *Am J Physiol* 245:F100–F109, 1983
51. SCHAEFFER JA, TROUTMAN SL, WATKINS MS, ANDREOLI TE: Flow dependence of fluid transport in the isolated superficial pars recta: evidence that osmotic disequilibrium between external solutions drives isotinic fluid absorption. *Kidney Int* 20:588–597, 1981
52. SCHMIDT U, DUBACH VC: Na,K-ATPase. Intracellular localization within the proximal tubule of the rat nephron. *Pflügers Arch* 330:265–270, 1971
53. PITTS RF: *Physiology of the Kidney and Body Fluids*. (3rd ed). Chicago, Year Book Medical Publications, 1974
54. SCHULTZ SG: Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by "flush-through." *Am J Physiol* 41:F579–F590, 1981
55. PALFREY HC, FEIT PW, GREENGARD P: cAMP-stimulated cation cotransport in avian erythrocytes. Inhibition by loop diuretics. *Am J Physiol* 238:C139–C148, 1980
56. GREGER R, SCHLATTER E: Properties of the basolateral membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney—a model for secondary active chloride transport. *Pflügers Arch* 396:325–334, 1983
57. PETERSEN OH, MARUYAMA Y: Calcium activated potassium channels and their role in secretion. *Nature* 307:693–696, 1984
58. BURNHAM C, JØRGENSEN PL, KARLISH SJD: Reconstitution of Na,K,Cl-cotransporter and a barium sensitive K-channel from luminal membranes of rabbit outer renal medulla (*abstract*). *Proc Reg Meeting IUPS, Jerusalem* p 101, 1984
59. JØRGENSEN PL: Regulation of the (Na^+ + K^+)activated ATP hydrolyzing enzyme system in rat kidney. I. The effect of adrenalectomy and the supply of sodium on the enzyme system. *Biochim Biophys Acta* 151:212–224, 1968
60. JØRGENSEN PL: The role of aldosterone in the regulation of (Na^+ + K^+)ATPase in rat kidney. *J Steroid Biochemistry* 3:181–191, 1972
61. HORSTER M, SCHMID H, SCHMIDT U: Aldosterone in vitro restores nephron Na-K-ATPase of distal segments from adrenalectomized rabbits. *Pflügers Arch* 384:203–206, 1980
62. MERNISSI GE, DOUCET A: Specific activity of Na,K-ATPase after adrenalectomy and hormone replacement along the rabbit nephron. *Pflügers Arch*, in press
63. DOUCET A, KATZ AI: Mineralocorticoid receptors along the nephron: [^{3}H]aldosterone binding in rabbit tubules. *Am J Physiol* 241:F605–F611, 1981
64. VANDEWALLE A, FARMAN N, BENCSATH P, BONVALET JP: Aldosterone binding along the rabbit nephron: an autoradiographic study on isolated tubules. *Am J Physiol* 240:F172–F179, 1981
65. PETTY KJ, KOKKO JP, MARVER DC: Secondary effect of aldosterone on Na-K-ATPase activity in the rabbit cortical collecting tubule. *J Clin Invest* 68:1514–1521, 1981
66. WADE JB, O'NEIL RG, PRYOR JL, BOULPAEP EL: Modulation of cell membrane area in renal collecting tubules by corticosteroid hormones. *J Cell Biol* 81:439–445, 1979
67. SCHWARTZ GJ, BURG MB: Mineralocorticoid effects on cation transport by cortical collecting tubules in vitro. *Am J Physiol* 235:F576–F585, 1978
68. HIR ML, KAISSLING B, DUBACH UC: Distal tubular segments of the rabbit kidney after adaptation to altered Na- and K-intake II Changes in Na,K-ATPase activity. *Cell Tissue Res* 224:493–504, 1982
69. GARTY H, EDELMAN IS, LINDEMANN B: Metabolic regulation of apical sodium permeability in toad urinary bladder in the presence and absence of aldosterone. *J Membrane Biol* 74:15–24, 1983
70. MUJAIS SK, CHEKAL MA, JONES WJ, HAYSLETT JP, KATZ AI: Regulation of renal Na,K-ATPase in the rat. Role of the natural mineralo- and glucocorticoid hormones. *J Clin Invest* 73:13–19, 1984
71. DOUCET A, KATZ AI: Short-term effect of aldosterone on Na,K-ATPase in single nephron segments. *Am J Physiol* 241:F273–F278, 1981
72. GEERING K, CLAIRE M, GÄGGELER HP, ROSSIER BC: Receptor occupancy vs induction of Na-K-ATPase and Na^+ transport by aldosterone. *Am J Physiol* 248:C102–C108, 1985
73. SCHIMKE RT, DOYLE D: Control of enzyme levels in animal tissues. *Ann Rev Biochem* 39:930–933, 1970